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ORIGINAL ARTICLE

Serum neurofilament light chain: a promising early diagnostic biomarker for hereditary transthyretin amyloidosis?

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Abstract

Background and purpose: Hereditary transthyretin amyloidosis (ATTRv) is a life-threatening disease caused by mutations in the gene encoding transthyretin (*TTR*). The recent therapeutic advances have underlined the importance of easily accessible, objective biomarkers of both disease onset and progression. Preliminary evidence suggests a potential role in this respect for neurofilament light chain (NfL). In this study, the aim was to determine serum NfL (sNfL) levels in a late-onset ATTRv population and evaluate whether it might represent a reliable biomarker of disease onset (i.e., 'conversion' from the asymptomatic status to symptomatic disease in *TTR* mutation carriers).

Methods: In all, 111 individuals harbouring a pathogenic *TTR* variant (61 symptomatic ATTRv patients and 50 presymptomatic carriers) were consecutively enrolled. Fifty healthy volunteers were included as the control group. Ella™ apparatus was used to assess sNfL levels.

Results: Serum NfL levels were increased in ATTRv patients compared to both presymptomatic carriers and healthy controls, whilst not differing between carriers and healthy controls. An sNfL cut-off of 37.10 pg/mL could discriminate between asymptomatic and symptomatic individuals with high diagnostic accuracy (area under the curve 0.958; $p < 0.001$), sensitivity (81.4%) and specificity (100%).

Conclusions: Serum NfL seems to be a promising biomarker of peripheral nerve involvement in ATTRv amyloidosis and might become a reliable, objective measure to detect the transition from the presymptomatic stage to the onset of symptomatic disease. Further longitudinal studies are needed to confirm such a role and determine whether it could equally represent a biomarker of disease progression and response to therapy.

KEYWORDS
ATTRv amyloidosis, biomarkers, disease onset, neurofilament light chain, NfL

Angela Romano and Guido Primiano contributed equally to this work.

INTRODUCTION

Hereditary transthyretin amyloidosis (ATTRv, v for 'variant') is an adult-onset, autosomal-dominant disease with variable penetrance, caused by pathogenic variants in the *TTR* gene, encoding the transthyretin protein. This rare disease results from the diffuse extracellular deposition of misfolded mutant *TTR* as insoluble amyloid fibrils in several organs and tissues, causing progressive, irreversible disruption of organ structure and function [1, 2].

Commonly involved organs include the peripheral and autonomic nervous system and the heart. Other organs and systems, such as the eye, kidney and gastrointestinal tract [3–5], may also be involved, leading to a life-threatening multisystemic disease with great heterogeneity in phenotypic presentation and disease course, and severe prognosis if left untreated.

Recently, the management of ATTRv amyloidosis has radically changed thanks to the development of several therapeutic options, potentially able to delay or even prevent disease progression but maximally effective when started early in the disease course [6–8]. Based on these premises, there is nowadays a clear need for easily accessible, reproducible biomarkers for both monitoring disease progression and detecting disease onset.

As well documented, in ATTRv amyloidosis, amyloid deposition starts long before the onset of symptoms [2]. Thus, potentially irreversible organ damage is already possible by the time the disease becomes clinically evident. This makes crucial the identification and validation of sensitive biomarkers able to detect disease onset at a subclinical stage, to effectively modify the disease's natural history and clinical outcome.

In the last few years, several reports have supported the role of neurofilament light chain (NfL) as a possible biomarker for subclinical disease in ATTRv amyloidosis [9–14]. Neurofilaments are major proteins of the neuronal cytoskeleton and are composed of three subunits: heavy, medium and light chains [15]. After a neuro-axonal injury, NfL is released in the extracellular space and is therefore detectable in cerebrospinal fluid and blood [16]. For this reason, NfL is emerging as a promising, both diagnostic and prognostic, disease biomarker in the field of neurology. However, NfL is not specific, as its levels increase in several neurological diseases, proportionally to the degree of axonal damage but regardless of the aetiopathogenesis [17, 18].

Since ATTRv amyloidosis is characterized by a progressive mainly axonal polyneuropathy, NfL may represent a reliable biomarker of disease progression and severity. Furthermore, preliminary data suggest that NfL may also represent a sensitive marker of disease onset, helping to discriminate still-asymptomatic *TTR* mutation carriers from early symptomatic patients [9–11, 14]. Raised levels of NfL may indeed occur even before symptom onset and evidence of detectable abnormalities on clinical examination and nerve conduction studies.

In this study, the aim was to evaluate the role of serum NfL (sNfL) as an objective, sensitive biomarker of peripheral nerve involvement in ATTRv amyloidosis.

MATERIALS AND METHODS

Study design and population

A cross-sectional study was performed involving different Italian centres with expertise in ATTRv diagnosis and management (Fondazione Policlinico A. Gemelli IRCCS, Rome; Policlinico Umberto I, Rome; Ospedale Sant'Andrea, Rome; Azienda Ospedaliera Universitaria Federico II, Naples; Azienda Ospedaliera Policlinico Universitario G. Martino, Messina; Centro per lo Studio e la Cura delle Amiloidosi Sistemiche, Fondazione IRCCS Policlinico San Matteo, Pavia; Istituto Neurologico Carlo Besta, Milan).

Blood samples were consecutively collected from both symptomatic ATTRv patients and presymptomatic *TTR* mutation carriers. Presymptomatic carriers were defined as carriers of a confirmed *TTR* pathogenic variant, with no symptoms or signs of multisystem involvement at enrolment [19]. Control samples were collected from healthy volunteers without any neurological, cardiological or autoimmune disease.

Demographic, genetic and clinical data were collected for both patients and presymptomatic carriers. All the enrolled subjects underwent a complete neurological and neurophysiological evaluation by an expert neurologist at the time of sample collection, including traditional nerve conduction studies and Sudoscan. For patients with evidence of polyneuropathy (neuropathic or mixed phenotypes), disease severity was evaluated using common neurological outcome measures, that is, the familial amyloid polyneuropathy (FAP) stage, the polyneuropathy disability (PND) score and the neuropathy impairment score (NIS). The presence of multisystemic involvement, especially cardiological impairment, was also investigated with instrumental and laboratory tests (e.g., transthoracic echocardiogram, cardiac scintigraphy with bone tracers, and serum cardiac biomarkers).

The study was approved by the Ethics Committee of Fondazione Policlinico Agostino Gemelli IRCSS (Prot. ID 4108) as the Coordinator Centre and then by the local Ethics Committees of all other involved centres. It was carried out according to the principles of the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants in this study.

Methods

Blood samples were collected into serum separator tubes, clotted for 30 min at room temperature and then centrifuged at 1000 *g* for 15 min. Serum aliquots were stored at −80°C until analysis.

Serum NfL concentration was measured using the Simple Plex™ cartridge-based assay on the Ella™ platform (ProteinSimple, San Jose, CA, USA), according to the manufacturer's instructions.

Statistical analysis

The sample was described in its clinical and demographic characteristics using descriptive statistics techniques. Qualitative variables

were described with absolute frequencies and percentages (%). Quantitative variables were summarized as mean ± standard deviation and as median and interquartile range (IQR). The *Z*-score method was used to identify sNfL outliers. Relevant outliers, defined as values with a *Z*-score greater than 3 or less than −3, were excluded from all statistical analyses to avoid bias.

For categorical variables, comparisons were performed by applying the chi-squared test or the Fisher exact test, as appropriate.

The normal distribution of quantitative data and homogeneity of variance were assessed with the Shapiro–Wilk test and Levene's test, respectively. The comparison of means between two independent groups was performed with the independent samples *t* test or the Mann–Whitney test, as appropriate. Comparisons between three independent groups were performed using the ANOVA or the Kruskal–Wallis test, as appropriate. Post hoc pairwise comparisons were carried out using the Dunn–Bonferroni post hoc method.

Receiver operating characteristic (ROC) curves were created by plotting the true-positive rate (TPR, or 'sensitivity') versus the false-positive rate (FPR, or '1 – specificity') across different cut-off points. The area under the curve (AUC) was calculated. Youden's *J* index was used to identify the optimal cut-off that best maximized the difference between TPR and FPR.

Finally, multiple linear regression analysis was run to predict sNfL levels from age, sex, disease duration and disease severity (evaluated by NIS).

Statistical analysis was performed using IBM® SPSS® Statistics version 25.0 (IBM Corp., Armonk, NY, USA). For all analyses, statistical significance was set at $p < 0.05$.

TABLE 1 Main demographic and clinical characteristics of our study cohort.

	ATTRv patients	Presymptomatic carriers	HCs
No.	61	50	50
Sex (M/F)	48/13	23/27	25/25
Age at sample collection (years)	69.83 ± 7.72; 72.00 (66.00–75.00)	51.48 ± 11.09; 47.00 (43.00–59.00)	51.68 ± 11.80; 52.50 (40.75–60.25)
Age at onset (years)	63.54 ± 10.65; 65.00 (57.00–71.00)	N/A	N/A
Disease duration (years)	6.29 ± 6.05; 5.00 (2.00–9.00)	N/A	N/A
Genotype			
• Val30Met	30 (49.2%)	25 (50.0%)	
• Phe64Leu	17 (27.9%)	14 (28.0%)	
• Ile68Leu	5 (8.2%)	3 (6.0%)	
• Val122Ile	4 (6.6%)	3 (6.0%)	
• Glu89Gln	2 (3.3%)	4 (8.0%)	N/A
• Ala109Ser	1 (1.6%)	1 (2.0%)	
• Ala120Ser	1 (1.6%)	0 (0%)	
• Val32Arg	1 (1.6%)	0 (0%)	
Phenotype			
• Mixed	48 (78.7%)		
• Neuropathic	7 (11.5%)	N/A	N/A
• Cardiopathic	6 (9.8%)		
Treatment at sample collection			
• Not treated	16 (26.2%)		
• *TTR* stabilizers	11 (18.0%)	N/A	N/A
• *TTR* gene silencers	34 (55.7%)		
sNfL (pg/mL)	74.00 ± 43.45; 68.50 (47.20–89.40)[a]	13.14 ± 9.92; 10.55 (5.69–18.03)	17.70 ± 8.44; 15.35 (12.28–23.63)

Note: Categorical variables are expressed as count and percentage (%). Numerical variables are expressed as mean ± standard deviation (SD), median and interquartile range (IQR).

Abbreviations: ATTRv, hereditary transthyretin amyloidosis; F, female; HCs, healthy controls; M, male; N/A, not applicable; No., number of enrolled subjects in each group; sNfL, serum neurofilament light chain.

[a]Reported values for sNfL refer to the ATTRv patients' group without outliers ($n = 59$).

RESULTS

Sixty-one symptomatic ATTRv patients and 50 presymptomatic *TTR* mutation carriers were enrolled, all of whom were of Italian ancestry. Fifty healthy volunteers were used as a control group. The main demographic and clinical features of the study cohort are summarized in Tables 1 and 2.

Two relevant outliers belonging to the ATTRv patients' group (a 73-year-old man and a 69-year-old woman, showing sNfL levels of 1053 and 702 pg/mL, respectively) were excluded from all the statistical analyses to avoid bias. The woman harboured a V30M variant, whereas the man carried a V122I variant, associated with a mixed phenotype in both cases. Specifically, the female patient had a long disease duration (12 years) and an aggressive course with the need for a percutaneous endoscopic gastrostomy for severe dysphagia, despite a moderate neuropathy disability (FAP stage II, PND score IIIa). Conversely, the male patient had a short disease duration (1 year) and a milder severity (FAP stage I, PND score II), yet a brain and spine magnetic resonance imaging revealed a cerebral small vessel disease and lumbar spinal stenosis.

A Kruskal–Wallis *H* test showed that there was a statistically significant difference in sNfL concentration between the three different groups ($\chi^2(2)=92.885$; $p<0.001$). The post hoc comparison revealed that sNfL levels were significantly higher in the ATTRv patients' group (mean value 74.00 ± 43.45 pg/mL, median 68.50, IQR 47.20–89.40) compared to both presymptomatic carriers (mean value 13.14 ± 9.92 pg/mL, median 10.55, IQR 5.69–18.03; $p<0.001$) and healthy volunteers (mean value 17.70 ± 8.44 pg/mL, median 15.35, IQR 12.28–23.63; $p<0.001$), whereas there was no significant difference between presymptomatic carriers and controls ($p=0.118$; Figure 1).

As regards the ATTRv patients' group, a statistically significant difference was detected stratifying them based on disease severity as scored by both the FAP stage and PND score (Table 3). Conversely, no statistically significant difference was found when stratifying ATTRv patients based on the genotype, phenotype or treatment at the time of sample collection (Table 3).

Receiver operating characteristic curve analysis was used to evaluate the ability of sNfL to discriminate the transition from the presymptomatic to the symptomatic stage of the disease (Figure 2a). The AUC of the ROC curve comparing symptomatic ATTRv patients with presymptomatic carriers was 0.958 (95% confidence interval [CI] 0.925–0.991, $p<0.001$). An sNfL concentration of 37.10 pg/mL could discriminate between these two groups with a sensitivity of 81.4% and a specificity of 100%.

The same sNfL threshold (37.10 pg/mL) was able to discriminate between presymptomatic carriers and the subgroup of ATTRv patients in the early symptomatic stage (PND score I) with a sensitivity of 63.2% and a specificity of 100% (AUC 0.897, 95% CI 0.813–0.981, $p<0.001$) (Figure S1a).

Similar results were obtained both comparing ATTRv patients with healthy controls (HCs) (AUC 0.937, 95% CI 0.892–0.983, $p<0.001$; sensitivity of 81.4% and a specificity of 98.0% for the sNfL cut-off value of 37.00 pg/mL; Figure 2b) and comparing the subgroup of PND I patients with HCs (AUC 0.842, 95% CI 0.724–0.961, $p<0.001$; sensitivity of 63.2% and a specificity of 98.0% for the same serum threshold of 37.00 pg/mL; Figure S1b).

Receiver operating characteristic curves were also created to evaluate whether it was possible to discriminate different stages of symptomatic disease. On comparing early symptomatic ATTRv patients (PND I) with more advanced disease stages characterized by motor dysfunction (PND ≥ II), the AUC was 0.835 (95% CI 0.722–0.949, $p<0.001$; Figure S2). An sNfL threshold of 57.70 pg/mL seems to be able to discriminate between these two groups with a sensitivity of 82.4% and a specificity of 73.7%.

Finally, multiple regression analysis was run in the subgroup of patients with evidence of a polyneuropathy (neuropathic or mixed phenotype; $n=53$) to predict sNfL from age, sex, disease duration and disease severity (evaluated by NIS). Regression analysis showed that this model significantly predicted sNfL concentration ($F_{4,48}=4.865$, $p=0.002$, $R^2=28.8\%$), with the NIS score as the only statistically significant predictor ($\beta=0.470$, $p=0.001$), in contrast to age, sex and disease duration (all $p>0.05$).

TABLE 2 Main clinical neurological scales and scores for ATTRv patients with evidence of polyneuropathy (neuropathic or mixed phenotype), not including outliers.

ATTRv patients with evidence of PN ($n=53$)	
FAP stage (*n*)	
• I	32 (60.4%)
• II	20 (37.7%)
• III	1 (1.9%)
PND score (*n*)	
• I	19 (35.8%)
• II	13 (24.5%)
• IIIa	12 (22.6%)
• IIIb	8 (15.1%)
• IV	1 (1.9%)
NIS	60.09 ± 45.52; 59.75 (19.00–92.00)

Note: Categorical variables are expressed as count (*n*) and percentage (%). Numerical variables are expressed as mean ± standard deviation (SD), median and interquartile range (IQR).

Abbreviations: ATTRv, hereditary transthyretin amyloidosis; FAP, familial amyloid polyneuropathy; NIS, neuropathy impairment score (range 0–244); PN, polyneuropathy; PND, polyneuropathy disability.

DISCUSSION

In this study, the aim was to evaluate the role of sNfL as a possible reliable biomarker of peripheral nerve involvement in ATTRv amyloidosis.

In the last few years, progress in ATTRv treatment has radically changed the approach to the disease. Several diagnostic tests have been evaluated as potential biomarkers of both disease onset and

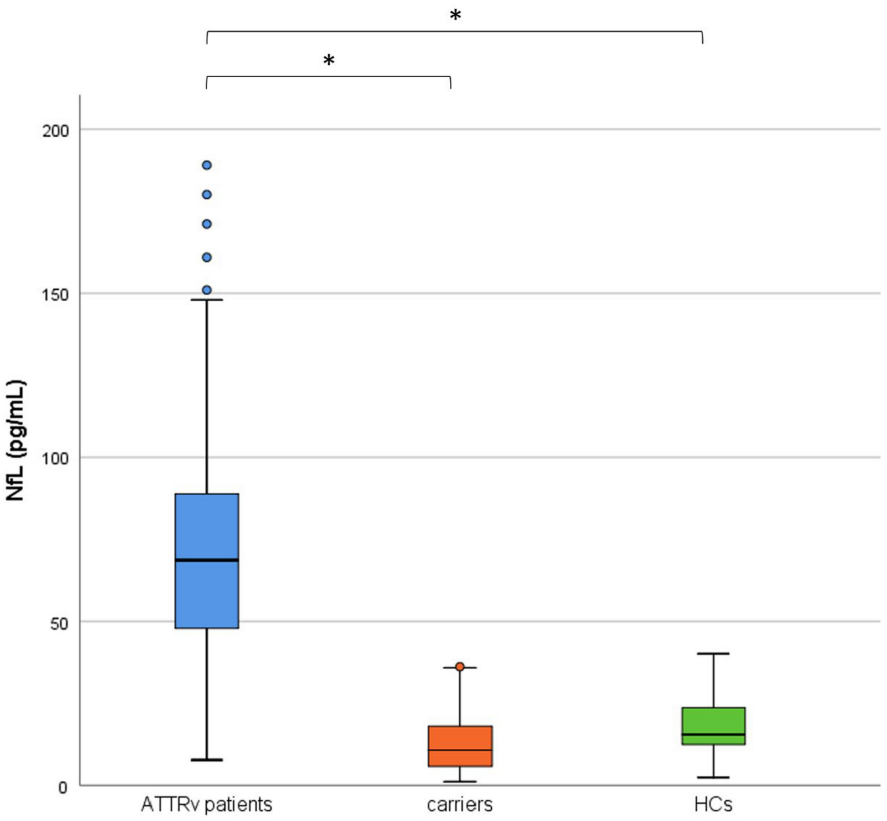

FIGURE 1 Serum NfL levels in the three different study groups: ATTRv patients, presymptomatic carriers and healthy controls (HCs). Each box represents the area between the first (Q1) and the third (Q3) quartile (interquartile range, IQR), with the lines inside the boxes representing the median values (Q2). Whiskers extend to the lowest and highest values within 1.5 times the IQR. *$p < 0.001$.

progression for prognostic purposes. Amongst the several proposed tools, the quantification of blood levels of NfL, a proven marker of neuro-axonal damage, has the clear advantage of being a reproducible, non-invasive, objective measure. Moreover, NfL can be used for longitudinal follow-up, although expensive and not yet widely available.

Given its neuron-specific nature, in the future NfL may gain a role as the 'neurological equivalent' of troponin [20]. However, it lacks specificity, as elevated levels have been reported in several diseases involving either the central or peripheral nervous system [17]. So far, the most robust data refer to patients with multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, frontotemporal dementia and parkinsonism [21–23], but growing evidence supports its potential role also in disorders involving the peripheral nervous system, including ATTRv amyloidosis [9–14].

Our cohort, including more than 100 either presymptomatic or symptomatic individuals harbouring a pathogenic *TTR* variant, is the second biggest so far described after the paper by Ticau et al. analysing the data emerging from the APOLLO trial [12].

Our findings confirm that sNfL levels are increased in patients with symptomatic ATTRv amyloidosis compared to both presymptomatic carriers and HCs (around 5- and 4-fold higher, respectively). Notwithstanding the different methods used (Ella in our study), our results are in line with previous studies showing plasma or sNfL levels 3–10 times higher in patients compared to still presymptomatic carriers and controls [9–14]. Interestingly, there was no significant difference when stratifying ATTRv patients based on the phenotype. This might suggest that even patients with a pure cardiac phenotype could have a subclinical neurological involvement.

Nowadays, several ultrasensitive immunoassay methods are available for quantifying NfL concentration in both cerebrospinal fluid and blood in the context of several neurological disorders. In the literature, the most used instrument is the single molecular array (SiMoA™, Quanterix). Still, other ultrasensitive methods such as the Ella instrument may be used as well [16]. Although serum/plasmatic NfL levels detected with Ella are significantly (around 17%–24%) higher than those measured by SiMoA, several reports suggest that these assays are equivalent and can both be used in routine clinical practice for longitudinal follow-up [16, 24–26]. In a study performed on patients with dementia, both assays proved indeed to be able to detect higher plasmatic NfL levels in patients than in controls, both allowing their discrimination with excellent diagnostic performance [25].

As concerns ATTRv amyloidosis, our study showed that an sNfL level of 37.10 pg/mL might help discriminate between presymptomatic and symptomatic individuals with extremely high diagnostic accuracy, a sensitivity of approximately 80% and a specificity of 100%. Such data suggest a potential role of NfL in recognizing the onset of symptomatic disease. Both the detection of a similar threshold (37.00 pg/mL) in differentiating ATTRv patients from the control group and the absence of a significant difference in sNfL levels between presymptomatic carriers and HCs support this hypothesis. The detection of such a threshold could thus represent a red flag for 'disease conversion', calling for more attention toward those carriers and pushing further investigation with more advanced diagnostic tests.

Previous studies aimed at defining an NfL threshold able to discriminate presymptomatic carriers from symptomatic ATTRv patients reported extremely variable values [10, 12, 14]. In fact, the

TABLE 3 The main comparisons in the group of ATTRv patients excluding outliers (n(total) = 59), stratifying them based on genotype, phenotype, disease severity or treatment at the time of sample collection.

	sNfL (pg/mL)	p	Pairwise comparisons
Genotype			
• Val30Met (n = 29)	74.00 ± 45.71; 67.80 (43.65–96.20)	0.879	N/A
• Non-Val30Met (n = 30)	74.00 ± 41.93; 68.65 (47.25–88.15)		
Phenotype			
• Mixed (n = 46)	80.60 ± 45.13; 71.15 (49.40–110.25)		
• Neuropathic (n = 7)	49.55 ± 26.90; 56.00 (18.20–71.60)	0.171	N/A
• Cardiopathic (n = 6)	51.93 ± 29.62; 51.25 (23.10–79.13)		
Disease severity, FAP stage			
• FAP stage I (n = 32)	62.54 ± 39.42; 55.75 (32.03–75.60)	0.005*	N/A
• FAP stage II (n = 20)	98.30 ± 44.61; 75.60 (62.95–136.00)		
Disease severity, PND score			
• PND score I (n = 19)	45.99 ± 26.79; 47.20 (18.70–67.80)		PND I versus II: p = 0.030*
• PND score II (n = 13)	86.72 ± 43.26; 72.10 (56.55–123.00)	0.001*	PND I versus IIIa: p = 0.008*
• PND score IIIa (n = 12)	93.72 ± 40.95; 75.15 (65.03–122.50)		PND I versus IIIb: p = 0.015*
• PND score IIIb (n = 8)	105.16 ± 51.73; 91.25 (56.85–157.75)		p > 0.05 for all remaining comparisons
Treatment at sample collection			
• Not treated (n = 15)	68.26 ± 54.21; 68.80 (22.30–89.40)	0.475	N/A
• Treated (n = 44)	75.96 ± 39.67; 68.30 (48.88–89.85)		

Note: The asterisk denotes statistically significant at the 0.05 level. Post hoc pairwise comparisons are reported when applicable.

Abbreviations: ATTRv, hereditary transthyretin amyloidosis; FAP, familial amyloid polyneuropathy; PND, polyneuropathy disability; N/A, not applicable; sNfL, serum neurofilament light chain (expressed as mean ± standard deviation; median and interquartile range).

specific cut-off may vary depending on the used immunoassay (SiMoA vs. Ella) and the specific sample (plasma vs. serum). To our knowledge, this is the first report on ATTRv amyloidosis using the Ella platform on serum samples. Compared to SiMoA, the Ella has the advantage of being a less expensive technique, and thus potentially more widely available. For this reason, standardized cut-off values obtained using this technique might have wider application in future clinical practice.

For the present study, a relatively large study cohort was studied for such a rare disease. In contrast with other studies, detailed clinical data were available for all enrolled patients and presymptomatic carriers. However, our cohort was quite heterogeneous, and also patients on treatment with specific disease-modifying therapies at the time of sample collection were included in the analysis. Nevertheless, no statistically significant differences were found between treated and untreated patients of our cohort. Previous evidence suggests indeed that NfL levels decrease after treatment initiation, yet they never return to baseline values [12].

Moreover, there was a clear significant age difference between patients on the one hand and carriers and controls on the other. This represents another bias since an age-dependent increase in NfL levels has already been documented [18]. For this reason, it would be important to define age-adjusted cut-off values. This is especially important for the elderly since they may be affected by concomitant diseases leading to neuro-axonal injury and subsequent unspecific rise in NfL levels. Nevertheless, our study proved that disease severity (evaluated by NIS) was the only significant predictor of sNfL concentration. This finding is in line with the paper by Maia et al., which demonstrated that the effect of disease severity on plasma NfL levels largely outweighs the age-related increase [10].

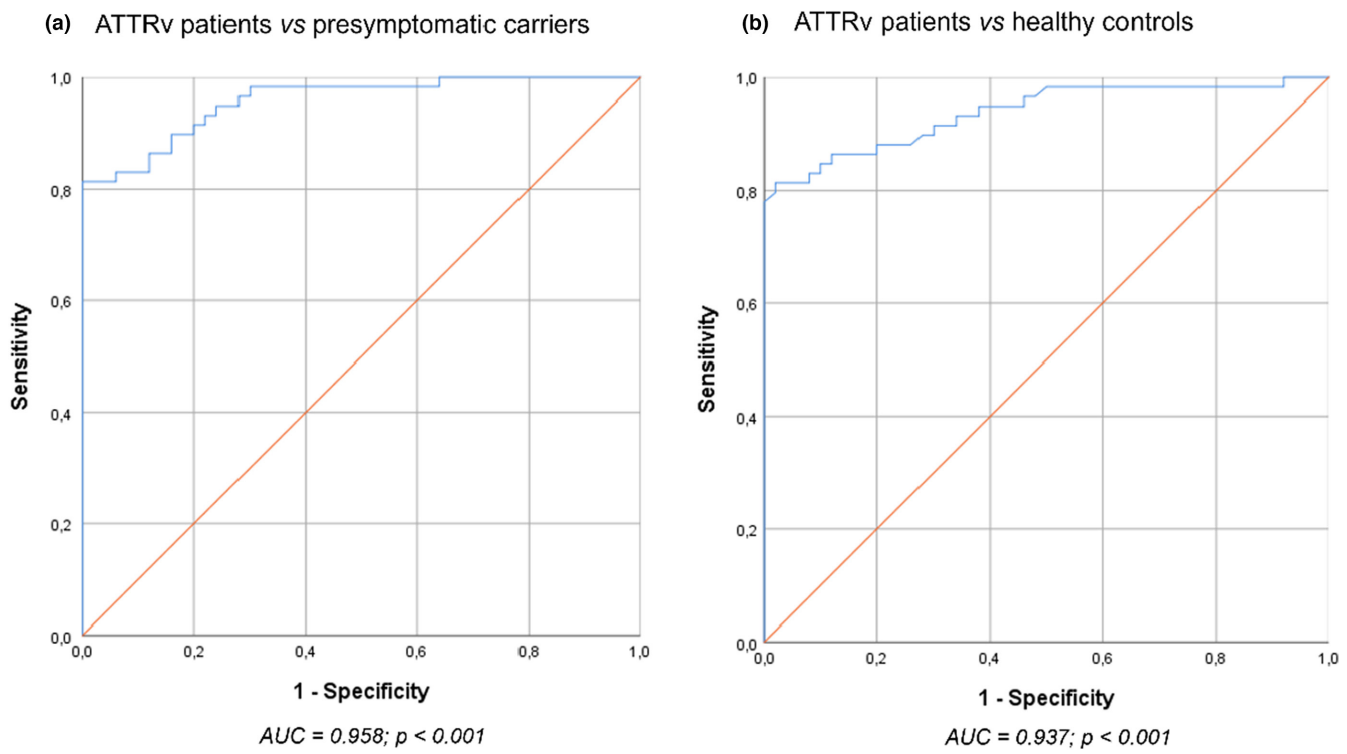

FIGURE 2 Receiver operating characteristic curves comparing the true-positive rate (sensitivity) versus the false-positive rate (1 – specificity) across different thresholds of serum NfL (sNfL) in differentiating ATTRv patients from either presymptomatic carriers (a) or healthy controls (b). The ROC curves and the corresponding AUC shows that sNfL has a good predictive ability to discriminate patients with symptomatic disease from both carriers and controls.

Another important limitation of our study is its cross-sectional design and the absence of a long-term follow-up so far. Further longitudinal collaborative studies are warranted to validate our data and define whether NfL might be used as a marker of both disease onset and response to therapy.

CONCLUSIONS

Neurofilament light chain is a promising biomarker in ATTRv amyloidosis and might have a role as an objective, sensitive predictor of disease conversion in presymptomatic *TTR* mutation carriers. A serum cut-off of 37.10 pg/mL (evaluated by Ella) could discriminate between still-asymptomatic carriers and symptomatic patients with high diagnostic accuracy, sensitivity and specificity. Longitudinal studies are needed to confirm these data and evaluate NfL's role as a marker of disease progression and response to therapy.

AUTHOR CONTRIBUTIONS

Angela Romano: conceptualization (supporting); methodology (supporting); data curation (equal); investigation (supporting); formal analysis (lead); resources (equal); writing—original draft (lead); writing—review and editing (equal). Guido Primiano: conceptualization (supporting); methodology (supporting); data curation (equal); investigation (lead); formal analysis (supporting); resources (equal); writing—original draft (supporting); writing—review and editing (equal). Giovanni Antonini: resources (equal); writing—review and editing (equal). Marco Ceccanti: resources (equal); writing—review and editing (equal). Silvia Fenu: writing—review and editing (equal). Francesca Forcina: resources (equal); writing—review and editing (equal). Luca Gentile: writing—review and editing (equal). Maurizio Inghilleri: resources (equal); writing—review and editing (equal). Luca Leonardi: resources (equal); writing—review and editing (equal). Fiore Manganelli: resources (equal); writing—review and editing (equal). Laura Obici: writing—review and editing (equal). Andrea Sabino: investigation (supporting); writing—review and editing (equal). Maria Ausilia Sciarrone: writing—review and editing (equal). Stefano Tozza: resources (equal); writing—review and editing (equal). Francesca Vitali: writing—review and editing (equal). Marco Luigetti: conceptualization (lead); methodology (lead); data curation (equal); supervision (lead); resources (equal); writing—original draft (supporting); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

AR received financial grants (honoraria and speaking) from Akcea, and travel grants to attend scientific meetings from Akcea, Alnylam, Pfizer and CSL Behring. SF received financial support from Alnylam, Pfizer and Akcea for participation in national and international meetings; participation in Advisory Boards of Akcea. FM received personal fees for scientific events from Alfa-Sigma, Alnylam Pharmaceuticals and Akcea Therapeutics, and received a travel grant to attend scientific meetings from CSL Behring. LO received speaking honoraria from Alnylam, SOBI and Pfizer and consulting honoraria from Alnylam, SOBI, Pfizer, Astra Zeneca, BridgeBio and Novo Nordisk. MAS received travel grants to attend scientific meetings from Sobi. ST received personal fees for scientific events from Alnylam Pharmaceuticals and Amicus Therapeutics, and travel grants to attend scientific meetings from Akcea Therapeutics. FV received travel grants to attend scientific meetings from Alnylam. ML received financial grants (honoraria and speaking) from Ackea, Alnylam, Sobi and Pfizer, and travel grants from Ackea, Alnylam, Sobi, Pfizer, Kedrion and Grifols. All other authors declare no disclosures.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Fondazione Policlinico Agostino Gemelli IRCSS (Prot. ID 4108) as the Coordinator Centre and then by the local Ethics Committees of all other involved centres. It was carried out according to the principles of the 1964 Declaration of Helsinki. Written informed consent was obtained from all participants in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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